Biocompatibility Evaluation of Poly(*N*-isopropylacrylamide)-based Hydrogels for Craniofacial Bone Regeneration

Tiffany N. Vo, Adam K. Ekenseair, Patrick P. Spicer, Brendan M. Watson, F. Kurtis Kasper, Antonios G. Mikos.
Rice University, Houston, TX, USA.

Statement of Purpose: Tissue engineering strategies involving injectable, *in situ* forming hydrogel scaffolds capable of mesenchymal stem cell (MSC) delivery show promise for regenerating complex craniofacial defects. Hydrogels based on Poly(*N*-isopropylacrylamide) (PNiPAAm) are particularly attractive since MSCs can be easily mixed with the polymer solution at room temperature, and subsequently be encapsulated and evenly dispersed within the insoluble network upon thermogelation above the lower critical solution temperature (LCST). Klouda et al. demonstrated these hydrogels enabled osteogenic differentiation of encapsulated MSCs *in vitro* [1]. However, PNiPAAm hydrogels have two shortcomings that limit their tissue engineering potential: syneresis and non-degradability. Non-shrinking PNiPAAm-based hydrogels have been recently developed by our laboratory by copolymerizing PNiPAAm with reactive moieties for tandem chemical crosslinking with polyamidoamine (PAMAM) crosslinkers [2]. The objectives of this study were (i) to fabricate non-shrinking, biodegradable hydrogels by copolymerizing the PNiPAAm-based macromers with pendant lactone rings to enable hydrolysis-dependent degradation via LCST modulation [3] and crosslinking with polyamidoamine (PAMAM) crosslinkers and (ii) to evaluate the *in vitro* cytocompatibility of the leachable and degradation byproducts and the *in vivo* biocompatibility of the injectable system in an orthotopic defect.

Methods: Thermogelling macromers (TGMs) were prepared with PNiPAAm, glycidyl methacrylate, acrylic acid and the hydrolyzable ring, dimethyl-γ-butyrolactone acrylate (DBA), via free radical polymerization by adapting the protocol as previously described [2]. Low molecular weight PAMAM crosslinkers were created using a simple polymerization following established protocols [2]. The cytocompatibility of TGMs and crosslinked hydrogels was assessed with a fibroblast cell line using leachable assays following previous studies [4]. Cell viability was quantified using Live/Dead reagents and fluorescence plate reader and normalized to controls. *In vivo* evaluation of two hydrogel formulations (n=7) was performed in an 8 mm rat calvarial critical size defect following established protocols [5]. After harvest at 4 and 12 weeks, samples were analyzed with microcomputed tomography (microCT), histology and histomorphometry for biocompatiblity, syneresis and mineralization.

Results: Rapid gelling, non-shrinking hydrogels were fabricated from the mixing of TGMs with the PAMAM crosslinkers, resulting in highly swollen gels. Extensive cytocompatibility testing of the TGM and hydrogel demonstrated that hydrogel system presented little cytotoxicity, and there were no significant effects of different hydrogel parameters on cell viability except at the highest polymer densities (Figure 1). Additionally, the hydrogels did not impede neotissue formation within the defect (Figure 2).

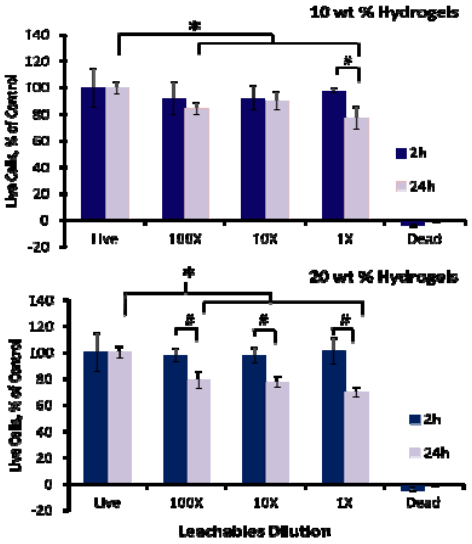

Figure 1. *In vitro* leachables cytocompatibility of dual gelling hydrogels for two TGM wt %. Cell viability for all groups was greater than 65%. * and # indicate statistical significance ($p<0.05$) within and between timepoints, respectively.

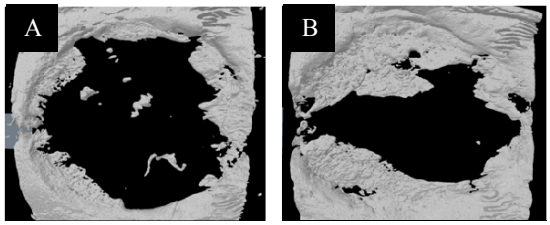

Figure 2. MicroCT images of 20 wt % hydrogels in the 8 mm rat calvarial defect at 4 (A) and 12 (B) weeks.

Conclusions: The results indicate that the presence of dual thermal and chemical crosslinking mechanisms can reduce hydrogel syneresis, which is beneficial for the incorporation and proliferation of cells. Furthermore, the hydrogel leachable products demonstrate *in vitro* cytocompatibility and the preliminary data suggest these hydrogels are biocompatible and potentially mineralize *in vivo*. In combination with MSCs, this *in situ* forming hydrogel system may provide a novel solution for localized and minimally invasive cell delivery for craniofacial bone regeneration.

References:

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